Hypofractionated radiotherapy for macroscopic canine soft tissue sarcoma: A retrospective study of 50 cas-es treated with a 5x6 gy protocol with or without metronomic chemotherapy

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Abstract: Wide surgical resection or a marginal/incomplete resection followed by full-course radiation therapy is the current standard of care for canine soft tissue sarcoma. The purpose of this retrospective, descriptive, bi-institutional study was to determine the effectiveness and toxicity of a hypofractionated 5 × 6 Gy protocol on macroscopic canine soft tissue sarcoma in terms of progression-free interval (PFI) and overall survival (OS), and to identify prognostic factors for patient outcome. Dogs with macroscopic soft tissue sarcoma irradiated with 5 × 6 Gy were eligible for the study. Progression-free interval and OS were compared with respect to different tumor and patient characteristics by the Kaplan-Meier method and multivariable Cox regression analysis. Fifty dogs with macroscopic disease were included. All dogs received the same radiation therapy protocol; part of the group (n = 20) received postradiation metronomic chemotherapy. Median PFI for all cases was 419 days (95% confidence interval (CI): 287-551) and median OS was 513 days (95% CI: 368-658). Dogs with tumors on the limbs had significantly longer PFI and OS, compared with head or trunk. Increasing tumor burden decreased OS. The addition of metronomic chemotherapy yielded a significantly longer OS (757 days (95% CI: 570-944) compared with dogs that did not receive systemic treatment (286 days (95% CI: 0-518), (P = 0.023)), but did not influence progression-free interval. Toxicity was low throughout all treatments. The 5 × 6 Gy radiation therapy protocol was well tolerated and provided long PFI and OS in dogs with macroscopic soft tissue sarcoma.

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Hypofractionated Radiotherapy for Macroscopic Canine Soft Tissue Sarcoma: A Retrospective Study of 50 Cases Treated with a 5x6 Gy Protocol with or without Metronomic Chemotherapy

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Abstract

Wide surgical resection or a marginal/incomplete resection followed by full-course radiation therapy is the current standard of care for canine soft tissue sarcoma. The herein presented results of a retrospective, cross-sectional, bi-institutional study describe the effectiveness and toxicity of a hypofractionated 5x6 Gy protocol on macroscopic canine soft tissue sarcoma in terms of progression-free interval and overall survival, identifying prognostic factors on outcome.

Dogs with macroscopic soft tissue sarcoma irradiated with 5x6 Gy were eligible for the study. Progression-free interval and overall survival were compared with respect to different tumor and patient characteristics by the Kaplan-Meier-method and multivariable Cox-regression analysis. Fifty dogs with macroscopic disease were included. All dogs received the same radiation therapy protocol; part of the group (n=20) received post-radiation metronomic chemotherapy. Median progression-free interval for all cases was 419 days (95%CI:287-551) and median overall survival was 513 days (95%CI:368-658). Dogs with tumors on the limbs had significantly longer progression-free interval and overall survival, compared with head or trunk. Increasing tumor burden decreased overall survival. The addition of metronomic chemotherapy yielded a significantly longer overall survival (757 days (95%CI:570-944) compared with dogs that did not receive systemic treatment (286 days (95%CI:0-518), (p=0.023)), but did not influence progression-free interval. Toxicity was low throughout all treatments. The 5x6 Gy radiation therapy protocol was well tolerated and provided long progression-free interval and overall survival in dogs with macroscopic soft tissue sarcoma.

Introduction

Soft tissue sarcomas refer to a group of malignant tumors that arise from mesenchymal cells and can occur in any part of the body, most commonly in the extremities and trunk. Although canine soft tissue sarcomas present with wide variations in subtypes (fibrosarcoma, perivascular wall tumors, peripheral nerve sheet tumors, liposarcoma, myxosarcoma, malignant mesenchymoma)[1, 2], these tumors tend to have a similar biologic behavior, consisting of locally invasive growth and usually a low-to-intermediate metastatic potential. Conversely, other mesenchymal tumors also arising from soft tissues, including hemangiosarcoma, histiocytic sarcoma and rhabdomyosarcoma, have a more aggressive biologic behavior and a higher tendency to spread.[3] Surgery with or without full-course post-operative radiation therapy is the mainstay of treatment of canine soft tissue sarcomas.[2, 4-6] Adequate treatment yields long survival times ranging from 1416[3] to 2270 days[4, 5], with only 33% of the cases succumbing to the disease. However, analyses of prognostic factors showed that the extent of resection, initial tumor size, anatomical location, histological grade and clinical stage were predictive of outcome.[3-5] It is well documented that the extent of resection is associated with local control of soft tissue sarcoma.[6, 7] However, one study describes that some of these tumors resected without an adequate margin of normal tissue may only have a failure rate of about 30%.[8] Unfortunately, in some clinical situations, surgery cannot be used without unacceptable morbidity, or gross disease is left behind at resection in an attempt to spare critical normal structures or avoid amputation in the case of soft tissue sarcoma located on the extremities.

Hypofractionated radiation therapy protocols for macroscopic or microscopic canine soft tissue sarcomas are currently used in a true palliative (e.g. providing pain relief and improvement of dysfunction)[9, 10] setting for elderly patients or patients presenting with serious co-morbidities, but also due to financial restraint of owners or logistical restraint of treatment

72 machine availability.[11-13] The responses in the macroscopic setting using a 4x8 Gy weekly
73 protocol have been found to be rather short-lived with about 5 months of median time to
74 progression.[11] Traditional dose-intense chemotherapy as a single treatment modality has not
75 proven to be effective in local tumor control; nevertheless, the administration of metronomic
76 chemotherapy has recently shown to delay tumor recurrence after incomplete resection of
77 canine soft tissue sarcomas[14], most likely by inhibiting tumor angiogenesis and suppressing
78 regulatory T-cells.[15]
79 With these considerations in mind, this retrospective, cross-sectional, bi-institutional study
80 was conducted to assess the effectiveness of a previously published 5x6 Gy hypofractionated
81 radiation therapy protocol[13, 16-19], with or without post-radiation metronomic chemotherapy, in
82 dogs with macroscopic (including unresected or recurrent) soft tissue sarcoma, and to
83 evaluate tumor and treatment variables associated with local control and survival. It was
84 hypothesized that the hypofractionated radiation therapy protocol would result in good
85 tolerability and provide long progression-free interval and overall survival.

Materials and Methods

Patient and tumor characteristics

Medical records of dogs with soft tissue sarcomas admitted to the Division of Radiation Oncology of the Vetsuisse-Faculty, University of Zurich, and the Centro Oncologico Veterinario, Sasso Marconi, Bologna, between 2006 and 2013 were reviewed. Due to the retrospective nature of this study, reporters were aware of outcome at the time of data recording.

Dogs were included in the study if they had a soft tissue sarcoma that was diagnosed by means of histopathology, underwent a 5x6 Gy radiation therapy protocol in a macroscopic setting with or without post-radiation metronomic chemotherapy. Dogs were excluded if their tumor occurred in the oral or nasal cavity, if they had received previous chemotherapy and/or radiation therapy, or in the case of histopathological diagnosis demonstrating histiocytic sarcoma, angiosarcoma or rhabdomyosarcoma, due to the documented different biological behavior.[3, 11, 20] Each dog had previously been evaluated through medical history, physical examination, routine blood tests, thoracic radiographs and other appropriate imaging studies. Information retrieved from all medical records included: signalment (age, sex, breed), concurrent diseases, presentation (primary versus recurrent), number of previous surgeries, involved sites, tumor size, histology, histological grade, clinical stage, treatment modality (electron versus photon plan), response to radiation therapy, time to disease progression, treatment-related complications, time and cause of death, and date of last follow-up visit. Tumor site was classified as head (excluding oral and nasal cavity), trunk, and extremities. Pathologic diagnoses were made by experienced pathologists (Dipl. ECVP) at the time of treatment according to currently accepted criteria.[21] Histological grade was based on a scale of 3 (low, intermediate, high grade).[3] The size of the tumor before radiation therapy was evaluated by caliper measurement from physical examination, radiography, and/or computed

tomography (CT), depending on anatomical site and clinicians' and/or owners' preferences. Tumor volume was calculated by the rotational ellipse method (max. height x max. width x max. length x $\pi/6$). Staging workup for regional and distant disease included fine-needle aspiration of the regional lymph node, thoracic radiography and abdominal ultrasound, and/or total body CT, depending on clinicians' and/or owners' preferences. Stage was established with the modified TNM staging system.[22]

Treatment

All dogs were treated with external beam megavoltage radiation therapy. Radiation was delivered with a 6 megavolt (MV) linear accelerator (Dynaray LA20; ABB, Clinac DMX or Clinac iX, Varian, Palo Alto, USA) using either photons (3-dimensional conformal radiation therapy, 3DCRT) or electrons, depending on tumor size and location. Treatment planning was performed on the basis of CT for photon plans or by hand calculation for electron plans. For treatment planning, the Eclipse External Beam Planning system version 8.1 or 10.0 (Varian Oncology Systems, Palo Alto, CA) was used, applying the pencil beam convolution- (version 8.6.14) or AAA-algorithm (10.0.28). Dogs were under general anesthesia for planning-CT and daily treatment, and – if treated with photon plans - immobilized in an individually shaped vacuum cushion (BlueBag BodyFix, Elekta AB, Stockholm, Sweden). Dogs with tumors in the head area were additionally immobilized with a custom-made bite block.[23] The GTV (gross tumor volume) was delineated using co-registered or in parallel viewed contrast-enhanced CT images, and CTV (clinical target volume), accounting for subclinical microscopic disease extension of 2-3 cm (presumed local infiltration) as well as positive regional lymph nodes), was defined. The CTV-margin was then extended three-dimensionally by 2 mm (for image-guided photon treatment), or 4-6 mm (for non-guided photon treatment)

135 to define the planning target volume (PTV), accounting for internal physiologic movements,
136 patient motion, and setup uncertainties. Organs at risk were segmented as applicable.
137 The recommendations for specifying dose and volumes as proposed for veterinary
138 medicine were adhered to as proposed in the corresponding literature.[24-26] For photon
139 plans, the prescribed dose was 30 Gy at the ICRU reference point[25], delivered in 5
140 fractions of 6 Gy applied twice per week, resulting in an overall treatment time of 2.5
141 weeks. Treatment was performed isocentrically with bolus and wedges to ensure dose
142 homogeneity for photon treatments. For tumors treated with electron fields, the
143 according fields were applied at 100 cm source surface distance (SSD) and field sizes
144 as well as energies were chosen to adequately cover the tumor with the additional
145 suspected margin of infiltration of 2-3 cm. The 90% isodose line was chosen to
146 encompass this target volume and for dose normalization.[27]
147 Radiation-related toxicity was graded according to the Veterinary Radiation Therapy
148 Oncology Group (VRTOG) scheme at each treatment, and 2-3 weeks after having
149 completed the protocol, thereafter monthly rechecks were scheduled.[28] Specific
150 attention was paid to wound-healing complications, in-field fractures, vascular
151 complications, and second malignancies. The recommendation for use of metronomic
152 chemotherapy was based on the judgment of the clinicians managing the cases and on
153 owners' preferences, and consisted of oral administration of thalidomide (Thalidomide
154 CEG, Celgene srl, Milan, Italy; 1-2 mg/kg/per day), piroxicam (Feldene, Pfizer, Rome,
155 Italy; 0.3 mg/kg/per day) and cyclophosphamide (Endoxan, Baxter, Rome, Italy; 7
156 mg/m^2/every other day). All drugs were re-formulated and administered to the nearest
157 5 mg.
158
159 *Outcome and follow-up*

160 Outcome information was obtained by means of medical records or communication with
161 referring veterinarians and owners at the end of the study period. Follow-up care included a
162 medical history and physical examination at progressive intervals beyond treatment. Response
163 data was noted according to the RECIST criteria for dogs.[29] Complete remission was defined
164 as the disappearance of all target lesions. Partial response (PR) was defined as a reduction of
165 at least 30% in the sum of diameters of target lesions from baseline. Stable disease (SD) was
166 defined as < 30% decrease or >20% increase in sum of diameters of target lesions from
167 smallest sum while on treatment. Progressive disease (PD) was defined as an increase in the
168 sum of diameters of target lesions by at least 20% over the size present at entry on study, or
169 the appearance of new lesions. Responses were required to last for at least 28 days. Follow-up
170 imaging was individualized based on baseline risk, symptoms, and clinicians' and/or owners'
171 preferences, and included thoracic radiographs and abdominal ultrasound or total body CT.
172
173 *Statistical analysis*
174 Statistical evaluation was performed together with a biostatistician (M.R.) and computed with
175 a commercial statistical software package (IBM® SPSS® Statistics, Version 22). Description
176 of quantitative data characteristics, other than progression-free interval (PFI) and overall
177 survival (OS), is given by mean (± SD), unless otherwise specified. For qualitative
178 characteristics absolute and relative frequencies were provided. Association of discrete patient
179 or tumor specific variables on the addition of adjuvant metronomic chemotherapy was
180 evaluated with the Chi-square-test or logistic regression. For logistic regression odds ratio
181 (OR) together with the corresponding 95%CI(OR) were computed.
182 PFI was defined as the interval between the first fraction of radiation therapy to measurable
183 progression of disease, while OS was defined as the interval between the first fraction of
184 radiation therapy and death. Dogs that were still free of progression at the time of data

evaluation were censored. For OS, all deaths were considered events and dogs that were still alive at the time of data evaluation or lost to follow-up were censored. The Kaplan-Meier survival analysis was followed by logrank or Breslow-Gehan-Wilcoxon tests. In case the survival curves did not cross the logrank test was applied. Otherwise the Breslow-Gehan-Wilcoxon test was used. The multivariable Cox-regression analysis was used to determine whether the following factors were significantly associated with PFI or OS: presentation (primary versus recurrent), number of previous surgeries, involved sites (head and neck versus trunk versus extremities), tumor size, histology, histological grade (1 versus 2 versus 3), regional lymph node metastasis (present versus absent), distant metastasis (present versus absent), medical treatment (yes versus no). Survival estimates are presented as median with the corresponding 95% confidence intervals (95%CI). In case median survival was not reached, the mean survival with the corresponding 95%CI was reported. For Cox-regression hazard ratio (HR) together with the corresponding 95%CI(HR) were computed. For factors not only the global p-value but also the pairwise Bonferroni-corrected post-hoc tests were reported. A p-value of <0.05 was considered significant for all analyses. For between levels comparisons of three-level factors within Cox-regression the Bonferroni correction was applied to significance $0.05/3=0.016$ and tendency $0.1/3=0.03$ levels to perform the pairwise Bonferroni-corrected post-hoc tests.

Results

Patient and tumor characteristics

Fifty dogs fulfilled the inclusion criteria for this study, of which 21 were female (15 spayed) and 29 were male (9 neutered). The dogs were of various pure (n=36) and mixed breeds (n=14) and a total of 19 breeds were represented. The ages ranged from 4-16 years with a mean of 9.8 (± 2.7) years. The weight ranged from 8-52 kg, with a mean of 29.3 (± 10.4) kg.

Of the 17 (34%) dogs that had previous surgeries, 9 (18%) had one and 8 dogs (16%) had 2. Pretreatment tumor volumes ranged from 0.06 to 491 cm^3, with a mean of 118.3 cm^3 (± 125). Six tumors (12%) were located on the head, 5 (10%) on the trunk, and the remaining 39 (78%) on the limbs. Of the 50 cases, 19 tumors (38%) were histologically described as perivascular wall tumors (including hemangiopericytoma, spindle cell sarcoma, myopericytoma), 13 (26%) as peripheral nerve sheet tumors, 8 (16%) as fibrosarcoma, 7 (14%) as undifferentiated/anaplastic sarcoma, 2 (4%) were myxosarcomas, and 1 (2%) was a liposarcoma. Histological grade was given in 42 cases (84%) with 52% grade 1 (n=26), 26% grade 2 (n=13), and 6% grade 3 tumors (n=3). For these dogs the modified TNM staging was applied and revealed 40 tumors to be of stage I, and 2 of stage II. For the remaining 8 cases, the histological samples were not available and grade could not be retrieved. None of the dogs presented with either regional or distant metastasis.

Treatment protocol, side effects

Thirty (60%) dogs were treated with single electron fields with energies ranging from 12-15 MeV, and 20 cases (40%) had a 3D-conformal photon plan. Acute side effects were assessed in 42 cases and consisted of grade 0 toxicity (33.3%) in 14 dogs, grade 1 toxicity (64.3%) in 27 dogs (erythema, dry desquamation, alopecia or epilation) and grade 2 toxicity (2.3%) in one dog (patchy, moist desquamation, without edema). Late effects were assessed in 37 cases with no toxicity (67.6%) in 25 dogs, and grade 1 toxicity (32.4%) in 12 dogs (leukotrichia). Twenty dogs received additional metronomic chemotherapy. Neither the histological grouping (p=0.067) nor tumor location (p=0.245) nor volume (p=0.232) was significantly relevant for the adjunction of chemotherapy. However, the proportion of dogs receiving metronomic chemotherapy decreased with increasing age. Elderly dogs received metronomic

chemotherapy less often (OR: 0.713 (95%CI:0.551-0.923), p=0.01) than younger dogs, with a 29% of administration reduction for each additional year of age.

Response to treatment

Follow-up time for censored cases (n=17) was 561 days (95%CI:482-640, range 87-929 days). Of the 33 animals that were known to have died, 15 (45.5%) died of tumor-related causes and 18 (54.5%) died of tumor -unrelated causes. At the end of radiation therapy, all (100%) dogs were evaluated for response. There were 5 (10%) complete responses, 10 (20%) partial responses, and 35 (70%) stable diseases. At 3-month re-evaluation, 41 (92%) dogs were assessed for response, and there were 6 complete responses, 13 partial responses, 20 stable diseases, and 2 progressive diseases. At 6-mont re-evaluation, 32 (64%) dogs were assessed for response, and there were 8 complete responses, 7 partial responses, 13 stable diseases, and 4 progressive diseases. Median progression free interval for all cases was 419 days (95%CI:287-551). Tumor location significantly influenced PFI, with tumors on the limbs (median PFI: 466 days (95%CI:321-611)) faring significantly better than tumors on the head or on the trunk (median PFI: 110 days (95%CI:0-238) and 203 days (mean, median not reached (SE=31 days (95%CI:142-264), respectively) (p=0.021);); (head vs. trunk (p=0.421, ns), head vs. limb (p=0.004, significant), trunk vs. limb (p=0.409, ns) after Bonferroni correction) (Figure 1).

The number of previous surgeries also significantly influenced PFI. Dogs having had >1 prior surgery had the shortest PFI (105 days (95%CI:0-349)), whereas dogs with no prior surgery or with one prior surgery were free of progression for a median of 420 days (95%CI:285-556) and 536 days (95%CI:92-980) (p=0.003); (no surgery vs. one surgery (p=0.579, ns), no surgery vs. > 1 surgeries (p=0.02, tendency), one surgery vs. > 1 surgeries (p=0.027, tendency)), respectively. Histology (p=0.080), tumor grade (p=0.167), radiation therapy

modality (p=0.585), age (p=0.296), and pretreatment volume (p=0.658) did not significantly influence PFI.

Median overall survival was 513 days (95%CI:368-658). Regarding histology, a significantly shorter survival in the fibrosarcoma group versus all other histologies was found (median 190 days (95%CI:38-343)) (p<0.001); grade did not influence survival (p=0.922). However, location was documented to be a significant prognostic factor: dogs with tumors on the limbs survived longer (579 days (95% CI:452-706) than those with tumors on the head (195 days (95%CI: 51-339)) and on the trunk (190 days (95%CI: 57-323)), (p=0.031); (head vs. trunk (p=0.366, ns), head vs. limb (p=0.038, ns), trunk vs. limb (p=0.015, significant) after Bonferroni correction) (Figure 2).

No association was found for tumor grade (p=0.631), treatment modality (p=0.735), or age (p=0.412), however, increasing tumor volume decreased OS (HR 1.004 (95%CI:1.001-1.007), p=0.005). The number of previous surgeries showed no significant association with OS.

The addition of metronomic chemotherapy did not influence the duration of PFI, however, dogs receiving metronomic chemotherapy had a significantly longer OS (757 days (95%CI:570-944) compared with dogs that did not receive systemic treatment (286 days (95%CI:0-518), (p=0.023)) (Figure 3). Interestingly, 3 dogs receiving metronomic chemotherapy that achieved partial response (n=2) or stable disease (n=1) at the end of radiation therapy obtained complete response at 6-month re-evaluation, and 1 dog that had stable disease at the end of radiation therapy obtained partial response at 6-month re-evaluation.

Discussion

The present study is based on a large number of dogs with soft tissue sarcoma with macroscopic (unresected or recurrent) disease treated with a hypofractionated 5x6 Gy radiation therapy protocol. For several reasons, such as financial or logistical restraints of the owners, co-morbidities of the patients amongst others, there seems to be a demand for non-curative approach for the treatment of canine soft tissue sarcoma. In a previous study, a 4x8 Gy palliative protocol for macroscopic disease revealed short progression free interval of 5.2 months and overall survival of 10.3 months.[11] The progression free interval and overall survival from the current study population were higher, with progression free interval of 13.9 months and overall survival of 17.1 months. Limb tumors fared significantly better with progression free interval of 15.5 months regardless of tumor burden, histology or grade. The general finding of long progression free interval and overall survival was surprising, as a satisfactory outcome of radiation therapy alone for soft tissue sarcoma is not usually expected, due to the rather low radioresponsiveness of sarcomas. Also, although the great majority of dogs obtained stable disease at the end of radiation therapy, 10% and 20% of the treated cases obtained complete responses and partial responses, respectively. Interestingly, the response rate further improved over time, highlighting that neoplastic cells keep dying off for months after the end of radiation treatment or respond slowly to metronomic chemotherapy.

The commonly used term “canine soft tissue sarcoma” is used for tumors with similar biologic behavior.[2, 30] However, while not described for surgical or multimodal curative approach[3-5], tumors on the limbs might respond better to hypofractionated protocols[12], or the limb location displays simply less discomfort for the patient.[11] Furthermore, this result may be attributable to the fact that the majority of limb soft tissue sarcomas are histologically described as perivascular wall tumors.[1] Compared to anaplastic sarcomas, it is known that they usually have less expansile growth with less tissue infiltration, low to no local aggressive

behavior with recurrence developing after a long latency, and low tendency to metastasize.[1, 31]

Management of recurrent tumors is often more difficult, and this study showed that more than one previous surgery in dogs presenting with macroscopic disease drastically reduced progression free interval to 3.4 months, while overall survival was not influenced. Multiple excisions may disrupt or contaminate the tissue planes, cause damage to existing vasculature with possible subsequent tissue hypoxia and could hypothetically lead to a selection pressure of cells with a higher malignancy in the recurrent tumor. Furthermore, recurrence of incompletely excised soft tissue sarcomas has been linked to histological grade [32], with a low recurrence rate to be expected in a microscopic disease setting of mostly low-grade soft tissue sarcomas.[12] The results of the current study also support this finding.

Larger tumor size is in general considered a poor predictive factor[2, 6], however, while most likely influencing the probability for complete surgical excision, size itself has not been identified as a prognostic factor in various studies.[30, 32, 33] In this study, increasing tumor volume decreased overall survival, perhaps indicating that larger tumors cause greater discomfort and prompt owners into the decision of euthanasia earlier.

Metronomic chemotherapy refers to the frequent administration of cytotoxic drugs at doses significantly less than the maximum tolerated dose, with no prolonged drug-free breaks, leading to an anti-angiogenic effect and immune-modulation.[34] Unlike traditional chemotherapy, the main targets of which are proliferating tumor cells, the main targets of metronomic chemotherapy are the endothelial cells of the growing vasculature of a tumor.[34] Although the use of dose-intense chemotherapy has been controversial in dogs with soft tissue sarcomas, the addition of metronomic chemotherapy has been recently described in the microscopic setting, leading to significantly prolonged disease-free intervals compared to a

nonrandomized control group.[14] In the current study, dogs receiving metronomic chemotherapy had a significant improvement in overall survival, whereas the progression free interval was not increased. Although encouraging, this finding needs to be interpreted cautiously. First, it should be borne in mind that OS may not be the best endpoint in veterinary oncology, as it may be influenced by tumor-unrelated factors, such as owners' financial concern or logistic issues, leading to premature euthanasia. Second, dogs receiving metronomic chemotherapy were younger than those that only received radiation therapy, and it may be possible that owners of younger dogs were more motivated in treating their animals than owners of elderly patients, which may have elected euthanasia sooner. Several reasons may explain why elderly dogs are less likely to receive chemotherapy, including the presence of concomitant diseases and the owner' unwillingness to accept possible negative effects of systemic treatment.

Interestingly, some dogs receiving metronomic chemotherapy experienced an improved response rate over time, with 3 cases obtaining complete response after partial response (n=2) or stable disease (n=1), and 1 case obtaining partial response after having achieved stable disease at the end of radiation therapy. Whether the addition of metronomic chemotherapy after radiation therapy is superior to radiation therapy alone needs to be tested in prospective trials. With this premise in mind, this study indicates that metronomic chemotherapy warrants further prospective investigation in canine soft tissue sarcoma to answer several questions. Indeed, the results obtained here did not allow identifying a subgroup of dogs that are more likely to benefit from metronomic chemotherapy.

Acute toxicity of the radiation protocol (mostly grade 0-1 skin reactions) was completely healed within two to three weeks after completion of treatment. During the time of follow-up, there was no evidence of late effects, which – due to the lower total dose and smaller fraction

size - are also expected to a lower degree than in the prior described 4x8 to 9 Gy protocols.[11, 12] Compared to Lawrence et al, where the „biologic equivalent dose“ (BED)[35] was calculated to be 117.3 Gy_3 (for late effects)[11] the comparable BED in this study is lower with 90 Gy_3. For the future, it could be considered to increase the dose per fraction of this protocol slightly, in order to improve response rate with a likely acceptable complication rate.

Also, toxicity of metronomic chemotherapy was mild as appropriate for a palliative approach, and allowed outpatient treatment. These findings are in accordance with previous studies.[11, 12, 14]

Due to the retrospective nature of this study, the use of archival data, and considering the well-known difficulty of proper clinical assessment of response of soft tissue sarcoma to radiation therapy, the authors may not have been able to describe the full range of improvement in these dogs and some of the information had to be gathered by telephone follow-up, possibly skewing the information by owner’s perception. Furthermore, different pathologists made the diagnoses and histological grade was only available in 84% of the cases. The modified stage grouping could only be applied in patients with known grade.[22] As most of the patients were of stage I disease and the prognostic significance of the staging system for soft tissue sarcoma has not been further investigated[2], the TNM was excluded from statistical description. The bi-institutionality of the study is of minor concern, as the treatment planning was either done remotely (all photon plans) or under the direct supervision (electron hand calculations) by the same radiation oncologist (CRB).

The standard of care for soft tissue sarcoma remains aggressive local therapy, which often involves a combination of surgery and full-course post-operative radiation therapy. With this treatment, median times to recurrence have been described with 700 to 798 days[4, 5] and were

hence considerably longer than the progression free interval of 419 days obtained with the much more simple protocol described herein. However, for dogs with non-resectable tumors, the herein described hypofractionated 5x6 Gy protocol is well tolerated and yields a satisfactory clinical outcome with negligible toxicity found in the clinically relevant observed time-frame. As with all hypofractionated protocols, a small risk of additional late toxicity with longer observation remains. Future prospective studies are warranted to determine whether metronomic chemotherapy after radiation therapy is beneficial for dogs with macroscopic soft tissue sarcoma.

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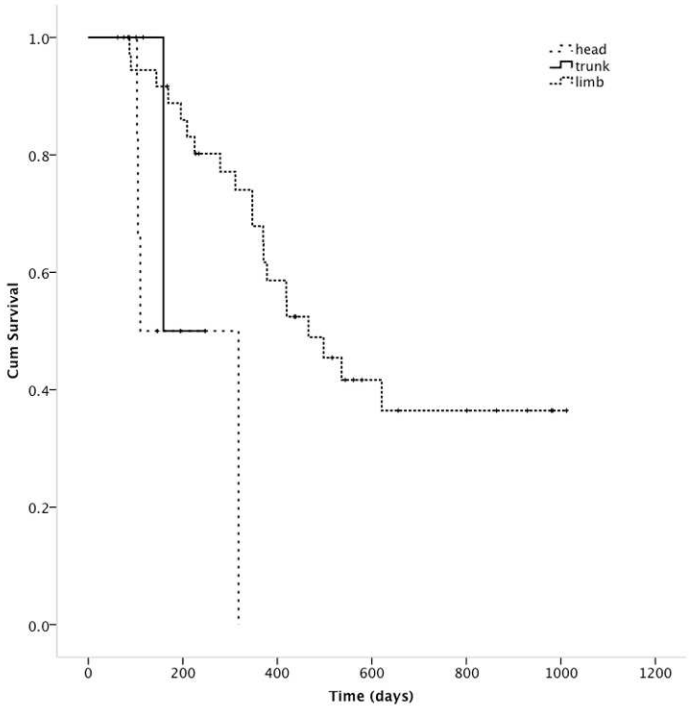
482

483 **Figure captions**

484 Figure 1: Progression-free interval for all cases grouped by tumor location (dotted line: limbs,
485 solid line: trunk, dashed line: head).

Figure 2: Overall survival times for all cases grouped by tumor location (dotted line: limbs, solid line: trunk, dashed line: head).

Figure 3: Overall survival times for patients treated without (dashed line) or with (solid line) metronomic chemotherapy.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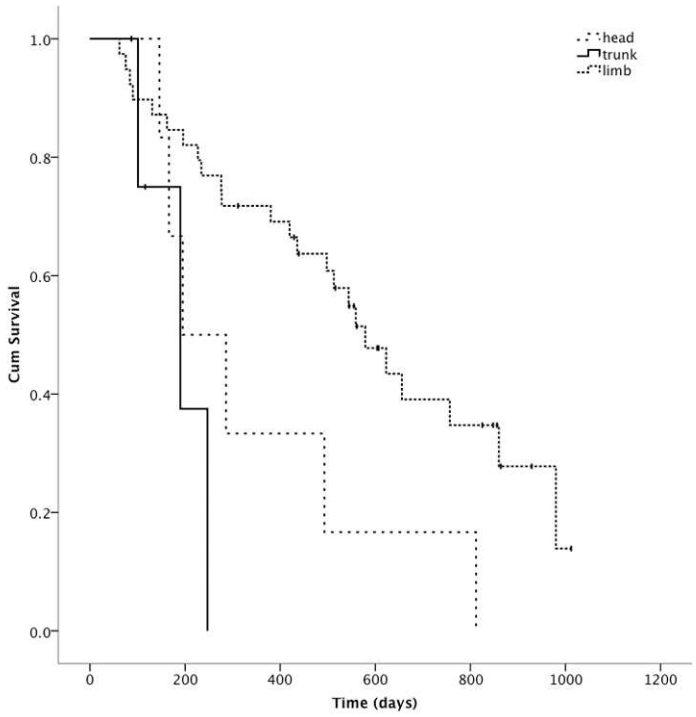
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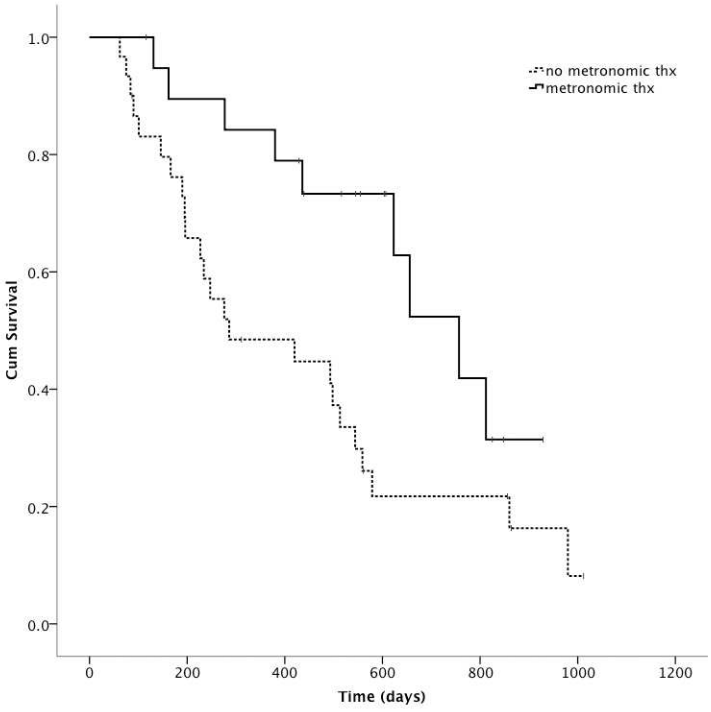
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